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PLANT DISEASE EPIDEMICS
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IDENTIFICATION SECTION

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HELMINTHOSPORIUM PROBLEMS

Supplement 228

October 15, 1954

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Plant Disease Epidemics and Identification Section serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

PLANT DISEASE REPORTER SUPPLEMENT

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PLANT DISEASE EPIDEMICS AND IDENTIFICATION SECTION

Horticultural Crops Research Branch Plant Industry Station, Beltsville, Maryland

Papers From The Symposium On

HELMINTHOSPORIUM PROBLEMS

presented at the Dallas meeting
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APPROACHES TO THE CLASSIFICATION OF HELMINTHOSPORIUM SPECIES

E. S. Luttrell

Although the taxonomic situation in Helminthosporium is no worse than in many other large genera of the Fungi Imperfecti, it is hardly necessary to state that problems exist or to argue for the need of further taxonomic study and revision. There has never been any attempt at a comprehensive treatment of Helminthosporium, not even of the species on grasses and cereals, the group of particular interest at the moment. A basis for study in this group has been laid in the excellent paper on graminicolous Helminthosporia by Drechsler (1). However, Drechsler did not include all of the species known to occur even in the United States at that time, and his work was published over thirty years ago.

At present there are slightly over 100 described species on grasses and cereals. These represent less than one-fourth of the total number in the genus. Certainly there are more than 450 names in Helminthosporium, and the number may approach 500. In addition to the graminicolous species there is a relatively small group which occur as hyperparasites on sooty mold fungi. There is also a large group of species which grow saprophytically on dead wood. Finally, there is another large group of species which are certainly or possibly parasitic on dicots or monocots other than grasses. Many of these are of economic importance. Helminthosporium papulosum Berg, cause of black pox of apples, for example, is now attracting attention in North Carolina and Georgia.

In a recent paper Hughes (4) expressed the opinion that the graminicolous species are incorrectly placed in Helminthosporium since they differ in what he considers to be fundamental characteristics from Helminthosporium velutinum Link ex Fr., the type species of the genus. H. velutinum is a member of the group occurring on old wood. There also appears to be some confusion as to the characters distinguishing Helminthosporium from other genera such as Napicladium, Brachysporium, and Curvularia. This indicates that questions of generic separations are involved, and it will be necessary to determine the relationships of the graminicolous species to other species in Helminthosporium and related genera. On the other hand, it might be inferred from Hughes' (4) study that the species on grasses represent a distinctive group which, temporarily, may be considered independently.

Compilation

There are no startlingly new approaches to the classification of Helminthosporium species. The only approach is persistent routine work of a nature which most find rather tedious. A first step toward a reorganization of the graminicolous species is an assessment of the present situation through a compilation of the literature dealing with these species. Even an alphabetical list of species would be helpful. Such a list must include all species of the genus. There are, for example, three fungi named Helminthosporium panici Two of these, H panici Van Overeem (15) and H. panici Sawada (10), are parasites on Panicum. The third species, and the one which has priority, is H. panici F. L. Stevens(13). Stevens' fungus is one of the hyperparasites on Meliola spp. A compilation of original descriptions of at least the graminicolous species would be desirable. A tentative key to species might also be attempted . Such a key would prove useful primarily in indicating possible relationships among species and in bringing together groups of similar species which should be subjected to comparative study. A key might also focus attention on the essential diagnostic characters. Species descriptions are still appearing in which no mention is made of important characters.

Type Collections

Since many of the original descriptions are completely inadequate, a study of type collections is essential. Many of the type collections are unobtainable, and some of those which can be located are nearly worthless. Even when the material is well preserved, it is difficult to determine such characters as type of germination and wall thickness of the dried conidia. Of course, it is impossible to determine pathogenicity. Even when type cultures have been preserved, these have often lost their virulence or ability to sporulate. Nevertheless, with these limitations, the study of type collections will prove profitable. The name Helminthosporium nodulosum B. & C. ex Sacc. (9), for example, was originally given to a fungus Curtis collected on Eleusine indica in South Carolina. More recently this name has been applied to a fungus pathogenic to Eleusine

coracana in Africa and India. The descriptions of Butler as cited by Sprague (12) and of Mitra and Mehta (6) are sufficient to indicate that the Indian fungus belongs among the group of species which might be termed the Helminthosporium oryzae-sacchari complex. H. nodulosum as represented in the type collection, which is preserved both in the Farlow Herbarium and at Kew, is, however, an entirely different fungus. It is very closely related to H. leucostylum Drechs. (1). This and other examples which could be cited illustrate the confusion in the application of names resulting from failure to consult readily available types.

Morphology

Thirdly, the range of variation within species and the proper delimitation of species should be determined by study of mass collections of field material. If, for example, the type of collections of Helminthosporium sorokinianum Sacc. ex Sorokin (11) and H. sativum P. K. & B. (7) are examined, differences in conidial shape and dimensions sufficient to distinguish the two species are apparent. However, study of a series of collections of fresh material has shown that there occur in the field specimens nearly identical with the type of H. sorokinianum, specimens nearly identical with the type of H. sativum, and specimens representing all intergradations between these two extremes. It now appears impossible to separate these two species satisfactorily. Parris (8) has published work of a similar nature on Helminthosporium sacchari (Van Breda de Haan) Butler.

Morphological studies of field material should be supplemented by cultural studies. Such studies should be made of the "wild types" as suggested by Miller (5) for Fusarium spp. It hardly seems necessary, so far as the pathologist is concerned, to attempt to classify the numerous cultural variants which have not been found in nature. The simplest method of maintaining isolates in their original form seems to be the method of soil culture revived by Miller (5) for the storage of Fusarium spp. A number of isolates of Helminthosporium sativum and H. victoriae Meehan & Murphy have been kept in tubes of soil at room temperatures without transfer for more than two years without loss of viability or original characteristics.

Conidia produced in culture show less variation than those formed under diverse conditions in nature. Cultural study, therefore, may assist in placing extreme forms which are sometimes found in field collections. Conidia in a collection of H. victoriae on oat culms made in South Georgia during hot, dry weather were straight, extremely thick-walled, and dark colored, and in these respects closely resembled the conidia of extreme forms of H. sativum. However, conidia produced in culture by isolates from this collection were typical of the thinner-walled, paler-colored conidia of H. victoriae.

In order to insure comparable results the method of producing conidia in culture should be standardized. Although the wounded-agar plate method has been satisfactory for inducing the sporulation in most species, conidia of some strains under these conditions are decidedly abnormal. The most satisfactory method tried is that proposed by Hansen and Snyder (3). Barley straw, coarsely ground in a Wiley mill, is placed with one cc. of propylene oxide in a quart jar which is then sealed for 24 hours. The gas-sterilized straw is scattered over the surface of plain water agar plates. The plates are inoculated at several points and incubated at a constant temperature of 20°C with exposure to normal day-light and darkness for two weeks. The conidia are comparable to those produced in nature. This method has been successful even with such difficult species as Helminthosporium gramineum Rab. It has failed only with H. siccans Drechs.

Cultural studies such as those of Elliott (2) in which factors of nutrition, temperature, light, and humidity are varied may provide information on the causes of variation in the field and assist in determining the relative value of various diagnostic characters. Tinline's (14) demonstration of sexual strains in Helminthosporium sativum suggests the further possibility of using genetic data in determining taxonomic relationships.

Pathogenicity

Finally, pathological studies must be made in conjunction with morphological studies. Since the pathologist is interested in species of Helminthosporium primarily as causes of disease in crop plants, a practical system of classification must express differences in pathogenicity among these forms. H. victoriae is of economic importance as a cause of a severe seedling blight of certain oat varieties. The pathologist, therefore, is under the practical necessity of recognizing this fungus. He may be inclined to separate this species on the basis of pathogenicity and to search for even minor differences that might be used to distinguish it from morpholo-

gically related forms. Wehmeyer (16) has made a valuable contribution in demonstrating that there is so little morphological difference among the Pleospora perfect stages of graminicolous species of Helminthosporium that all may be referred to the single species P. trichostoma (Fr.) Ces. & De Not. However, since these forms cause different diseases and in the conidial stage may be distinguished, even if by minor morphological differences, there is justification on utilitarian grounds for recognizing several species such as H. bromi Died., H. avenae Eidam, H. teres Sacc., and H. tritici-repentis Died., along with the corresponding perfect stage names. On the other hand, it may be convenient for the pathologist to regard as the same species forms on different hosts which cross-infect, even if considerable morphological variation exists.

In the classification of fungi of economic importance an attempt should be made to devise a system of the greatest practical usefulness, even if it is necessary to employ criteria different from those used in more obscure groups or groups of lesser importance. The extent to which tests for physiological characteristics should be used in recognizing species depends more on convenience than on any arbitrary decision as to whether characters should be considered morphological or physiological or what emphasis should be placed on either category.

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VARIATION IN PATHOGENCITY IN THE HELMINTHOSPORIUM BLIGHT ORGANISM OF OATS AND EVIDENCE FOR ITS RELATIONSHIP TO H. SATIVUM

H. R. Rosen

In 1946 evidence was presented in the Plant Disease Reporter (vol. 30 (10): 366-368) indicating that Helminthosporium blight of oats is not confined to Victoria derivatives. It was noted that Bond and some of its hybrids are not immune although they are highly resistant. This has been confirmed recently by W. C. Paddock (Cornell Agr. Exp. Sta. Memoir 315, 1953. p. 16).

In the fall of 1953 several single-spore isolates from a blighted Traveler plant (a Victoria derivative), when inoculated as a spore and mycelial suspension in sterile water on seedlings of a Bond derivative (R 22-66-53) and on several related derivatives, produced marked infections mostly confined to the distal ends of blades. These selections, when inoculated with other isolates, had previously shown a high degree of resistance to blight. On R 22-66-53, the infections often involved one-third to one-half of the blades and consisted at first of grayish-green collapsed tissue which within a few days turned brown. When such infected plants are placed in a moist chamber for 48 hours, the fungus covers the infected areas with a heavy mycelial growth readily observed on the surface of the leaves, much like that obtained on susceptible Victoria derivatives treated in a similar manner. When 100 seeds of this selection were inoculated with a spore and mycelial suspension, there was a 30.8 percent in stand compared with uninoculated seed, and this compared with 12.5 percent loss in stand of De Soto (a Victoria derivative) and an additional 14 percent loss in the latter from post-emergence blight. There was no post-emergence blight in R 22-66-53 although the total percentage of loss of plants was greater in this Bond derivative than in De Soto. The experiment involving seedling inoculations was repeated and essentially the same results were obtained. Thus there appears to be no doubt that there are strains or races of the blight organism that are capable of producing severe infections on oat varieties and selections that have no known Victoria inheritance.

It was also noted in the 1946 article previously cited that the variety Markton is susceptible, as well as Winter Turf 435-4. It may now be stated that the inclusion of Markton was probably an error occasioned by the presence of a relatively large number of rogues. In addition to oats, it was likewise noted that the barley varieties Fayette and Texan, when artificially inoculated, developed small dark brown or smallish spots on leaves of seedlings, much like those produced by Helminthosporium sativum but more restricted in size of spots.

In view of this evidence, plus the fact that spore measurements of conidia, color of spores, and polar germination of such spores, all indicated kinship to H. sativum, I have been reluctant to accept the name H. victoriae Meehan & Murphy and have recently reduced it to a varietal status (H. sativum var. victoriae (n.c.) Arkansas Agr. Sta. Bul. 533, 1953, p. 22).

In the earlier work of Dr. H. C. Murphy and his students (Science 104: 413-414, 1946; Phytopath. 37: 790-800, 1947) no mention is made of susceptibility to blight other than in Victoria derivatives. This was considered to be so highly specific for Victoria derivatives that when one of his students (Litzenberger, S. C. Iowa Agr. Exp. Sta. Res. Bul. 370, 1949) found susceptible plants in progenies of Mindo x Landhafer, "it was assumed that these were the result of natural crossing with plants susceptible to H. victoriae." Of course it could just as readily be assumed that there was no out-crossing and that there are strains or races of the blight organism fully capable of infecting plants that have no Victoria genes for susceptibility to blight.

That there are marked morphological differences in different isolates of the blight pathogen has been noted by almost all who have worked with this parasite for any length of time. Aside from saltations or mutations that are common in single spore cultures of many Helminthosporium species, differences have been noted in color and size of mycelium, in ability to produce conidia, and in color, measurements, and thickness of wall of conidia in the oat blight organism.

The difficulty experienced by Dr. R. W. Earhart in identifying the species of Helminthosporium that produces a culm rot on Southland oats (Phytopath. 43: 516-518, 1953) is to be expected so long as it is assumed that Helminthosporium blight is confined to Victoria derivatives. Southland represents a selection made by W. H. Chapman from the cross (D 69 x Bond) x Fultex (Florida Agr. Exp. Sta. Circ. S-18, 1950). Fultex represents a cross, Fulghum x Victoria, so that Southland presumably has both Bond and Victoria in its heritage. But Chapman found Southland to be resistant to blight, although he noted "some lodging due to a condition of

undetermined origin", and he also found it to be resistant to race 45 and related races of crown rust (Puccinia coronata). Since Murphy and his students have clearly shown a linkage between susceptibility to blight and resistance to race 45 of crown rust in Victoria derivatives, Earhart faced the almost impossible task of deciding what species of Helminthosporium incited culm rot on Southland. As this variety is resistant to both blight and race 45, it could not be considered as possessing typical Victoria genes, and without these, how could it be susceptible to an Helminthosporium culm rot unless one considered this rot to be caused by a wholly different species? Earhart partly solved this dilemma by identifying his isolates tentatively as H. sativum.

There is one other bit of evidence which leads me to conclude that the oat blight organism is related to H. sativum. Ito and Kuribayashi (Jour. Facult. Agr. Hokkaido Imp. Univ., Sapporo, 29: 85-125, 1931) described the perfect stage of H. sativum as Ophiobolus sativus, which they obtained in pure culture on rice-culm decoction agar. Apparently they did not collect this stage on natural hosts and I do not know of any such collections made elsewhere. Since the fall of 1945, a search has been made for the perfect stage of the oat blight organism on stubble and straw of varieties that had shown blight. Only one perithecium was found (University Farm, Fayetteville, Arkansas, January 7, 1947) which morphologically resembled O. sativus. It was found near the base of a leaf sheath of Traveler straw and was a dark, flask-shaped structure with no setae, measuring 384 x 240 microns. There were numerous asci and ascospores, the former appearing as worm-shaped threads, light yellowish-brown in color, rounded at the apex, tapering markedly at the base, with very short stipes, measuring 98-165 x 3.8 microns. The ascospores were much coiled or spiral shaped, exceedingly thin, colorless threads measuring 56-79 x 0.5 microns, easily broken into short segments by movement of the cover glass on the microscopic mount. These measurements derived from a single perithecium probably have little significance and are smaller than those given by Ito and Kuribayashi for O. sativus. But the flask-shaped perithecium without setae and the coiled ascospores which readily break into short segments are very distinctive characters found in O. sativus cultured from diseased barley and wheat in Japan.

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VARIATIONS WITHIN SPECIES OF HELMINTHOSPORIUM

Arnold J. Ullstrup

Variation in cultural behavior and virulence is a commonly observed phenomenon within the species of Helminthosporium actively pathogenic on corn. Robert (4) studied variability in cultural behavior and virulence among single-spore and hyphal-tip isolates of H. turcicum Pass. Variation in cultural behavior was expressed as colony color, type of mycelial growth, production of conidia, and growth rate. Wide variations were found in virulence. Specialization in parasitism was not studied. The variants were unstable in both cultural characters and virulence. Robles (5) reported two pathogenic races in H. turcicum based on their behavior on 11 varieties of corn in the seedling stage. In the writer's experience with a relatively large number of isolates of the fungus and many inbred lines, hybrids and open-pollinated varieties, seedlings have been uniformly susceptible; resistance was not expressed until plants were several weeks old. Specialization in parasitism among cultures of H. turcicum isolated from corn has never been observed by the writer. Inoculum taken directly from lesions of diseased leaf collections has always been highly virulent. In general the longer isolates have been in pure culture and the more frequently they have been transferred, the greater has been the variation in cultural behavior and virulence.

Despite the results with different lines of corn there is definite evidence of specialized parasitic races within H. turcicum. Mitra (2) observed the difference in parasitism between isolates from corn and those from sorghum. Lefebvre and Sherwin (1) pointed out that races within the species could be distinguished on the basis of their ability to attack corn, sorghum, Sudan grass, and Johnson grass. Two cultures from Sudan grass and one from sorghum were pathogenic on Sudan grass and sorghum, but failed to infect corn. Four of the cultures from corn infected Sudan grass, two did not, but all six were highly pathogenic on corn. A culture from Johnson grass was pathogenic only on its own host. Thus four distinct parasitic races were demonstrated among ten isolates.

Variation in cultural behavior has been shown in H. maydis Nisikado & Miyake (3). In the writer's experience this species is the most variable in cultural characters of those discussed here. The virulence of the isolates has remained stable, but detailed studies in this respect have not been undertaken. Specialization in parasitism has not been demonstrated within the species.

Helminthosporium carbonum Ullstrup appears to consist of two races that may be distinguished by their specialization in parasitism toward certain inbred lines of corn, their relative virulence, and symptoms incited on leaves (6). Race I is highly virulent and capable of complete destruction of suscepts. Susceptibility to this race is relatively rare as resistance is governed by a single dominant gene (7). Resistance and susceptibility to this race are demonstrable at all stages of host development. Race II is mildly virulent, and shows no pronounced specialization in parasitism, and host resistance is probably polygenic. Within both races cultural variants arise commonly in pure culture, but in Race I variation in virulence has not been observed.

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HELMINTHOSPORIUM DISEASES ON CORN

Arnold J. Ullstrup

There are three distinct and economically important diseases of corn in the United States incited by species of Helminthosporium.

Helminthosporium turcicum Pass. is the inciting agent of northern corn leaf blight. Conidia are olive-gray, spindle-shaped, often curved on one side, average 105 x 20 microns in size and have 1 to 9 septa. The hilum is conspicuous. No sexual stage of the fungus is known.

Symptoms appear as long elliptical, grayish green to tan spots, ranging up to 6 x 1.5 inches in size. Spores are produced on the surfaces of the lesions in damp weather. During severe outbreaks leaves may be nearly completely covered with lesions, leaving little green tissue. Observations in both naturally and artificially induced epiphytotics indicate that the disease does not attack ears, so that the possibility of seed transmission is very remote.

Northern corn leaf blight is found throughout most of the eastern half of the United States. Relatively cool weather accompanied by frequent heavy dews seems to favor spread and development of the disease. Warm, dry weather definitely checks it.

Resistance to infection by H. turcicum appears to be governed by a relatively large number of genes (1, 2). Resistance is not manifested until plants are several weeks old. Some of the sources of resistance which have been used as parental material are NC34, Mo21A, L97, Ky114, and C123. Very little resistance is present among the hybrids used commercially in the Corn Belt.

Helminthosporium maydis Nisikado & Miyake is the inciting fungus of southern corn leaf blight. Conidia are pale smoky-colored to olive-brown, average 90 x 115 microns in size, with 3 to 13 septa, and exhibit greater curvature than any other species attacking corn. The hilum is inconspicuous. Perithecia of the sexual stage, Cochliobolus heterostrophus Drechs., are black, beaked, and 0.4 to 0.6 mm in size. Asci measure 160-180 x 24-28 microns, and contain up to eight, but typically four ascospores. Ascospores are pale smoky-colored, 130-340 x 6-7 microns, 5-9 septate, in a helicoid arrangement within the asci. The sexual stage appears not to be abundant in nature, and its role in the progagation of the species may be unimportant.

Southern corn leaf blight occurs generally in eastern United States from the Ohio River Valley southward. The disease is favored by humid weather and appears to thrive at a somewhat higher temperature than is optimum for northern corn leaf blight.

The symptoms, which are readily distinguished from northern corn leaf blight, appear as elongated, tan lesions with generally parallel sides, that range up to 1-2 x 1/4-1/2 inches in size. The size, shape and color of the lesions may vary somewhat between different inbred hosts. Lesions on some inbred lines show a distinct, narrow, purple margin. Ears are not often infected.

Resistance, which is not expressed until plants are several weeks old, is manifested as small pale flecks where penetration has taken place. Resistance appears to be determined by several genes (3). Among the most resistant inbred lines of Corn Belt maturity are C103, Tr, Os426, and MoG.

Helminthosporium carbonum Ullstrup, the fungus inciting Helminthosporium leaf spot, appears to consist of two pathogenic races which are indistinguishable on the bases morphology, cultural behavior, or symptoms induced on corn ears. The two races are separable on the basis of leaf symptoms, virulence, and specialization in parasitism on inbred lines (5). Conidia are spindle shaped, straight to lightly curved, olivaceous brown, 2-12 septate, and average 62 x 13 microns in size. No sexual stage of the fungus is known.

Symptoms produced on suscepts following infection by Race I appear as tan, oval, zonate spots ranging up to 1 x 1/2 inch in size. The reaction on resistant hosts to infection is manifested as pale minute flecks about 1 mm in size, at points where the fungus has penetrated but its progress arrested. All parts of susceptible plants, including ears, may be attacked. The ears have a black, charred appearance. Race I is highly virulent and, under optimum conditions, suscepts may be killed before ear formation. As a consequence of ear infection the disease is readily disseminated through seed. Reaction of inbred lines to infection by Race I may be readily ascertained in the young seedling stage. Resistance is determined by a single dominant gene located on chromosome 1 (6). The disease incited by Race I is unique in that susceptibility is comparatively uncommon. Because of dominance of resistance, hybrids are not attacked unless two homozygous susceptible inbreds are crossed. Obviously, such combinations would not be made if the disease reaction of the inbred lines were known. Inbred lines known to be susceptible, and which are being or have been used in hybrid production, are K61, K44, Pr, and Mo21A.

Symptoms produced on plants in the field following infection by Race II appear as elongated, chocolate-brown spots ranging up to 2 x 1/2 inches in size. Symptoms on ears are identical with those incited by Race I. Symptoms on young seedling leaves are recognized as small elongated, tan spots. The disease incited by Race II is seldom encountered and consequently is of little importance. Race II is only mildly virulent and has never been observed to be as destructive as Race I. The mode of inheritance has not been studied, but a number of genes appear to be involved.

Other species of Helminthosporium have been reported as parasitic on corn, but these appear to be of very minor consequence. H. rostratum Drechs. has been found on corn in Mississippi (7), but it appears to be of rare occurrence and not destructive. H. sativum P. K. & B. has been cited as a pathogen of corn (4), but observations suggest that the disease must be very rare and certainly of no economic importance.

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